TRANSLATION NO. 326

DATE: July 1968

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE-T, Frederick, Md. 21701

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

U/C

THE CONTENT OF CORTICOSTERONE IN SUPRARENAL BLOOD OF RATS AT VARIOUS PERIODS AFTER IRRADIATION *

Problemy Endokrinologii i Gormonoterapii /Problems of Endocrinology and Hormone Therapy/, Vol IV, No 4, Moscow, 1958, pp 21-24.

M. A. Larina
T. S. Sakhatskaya

A number of works have been published in the literature on disturbances of neuro-endocrine regulation as the result of ionizing radiation applied to the organism. Irradiation induces an increase in the weight of the adrenals in animals, with reduction of the content of cholesterin and of ascorbic acid (14, 15, 12, 7, 5, 1 and others). However, the data on the content of corticosteroids in the blood and urine of irradiated animals and patients are somewhat contradictory.

It has been remarked (8) that there is an increase in the content of corticosteroids in the peripheral blood of dogs in the first 12-24 hours after irradiation, with subsequent reduction and a secondary rise prior to death. Similar changes were observed in monkeys (10, 11), and a dependence was demonstrated between the degree and duration of the increased concentration of 17-oxy-corticosteroids in the blood, and the dose of radiation. Determinations of corticosteroids in the blood of patients, whose adrenals have been therapeutically irradiated, have shown a reduction in the quantity of 17-oxy-corticosteroids immediately after irradiation, with a subsequent rise after about two hours (13). In these experiments, a connection was also noted between the amount of corticosteroid and the dose of radiation. X-ray therapy of the pituitary region in patients with Itsenko-Cushing's disease, to doses of 1000-15000 r, leads in the majority of cases (2) to elevation of the content of 11-oxysteroids in the blood. Upon increasing the dose to 1700-2500 r, the concentration in the blood declines.

---

* Presented at the scientific session of the All-Union Institute of Experimental Endocrinology on the problem of the "Pituitary", December, 1957.

In studying the secretion of the adrenals after irradiation of rabbits, by measuring the level of the corticosteroids in the blood flowing from the adrenals, it was shown (3) that in 3, 24, and 48 hours, and on the third and eighth days after irradiation, there was a reduction in the secretion of corticosterone and an increased secretion of hydrocortisone, aldosterone and compound X in the first three days after irradiation. However, in dogs studied at various periods after irradiation, lethal doses did not induce changes in the secretion from the adrenals, which is perhaps due to the different reactions of various species of animals to ionizing radiation (4).

We determined the content of the corticosteroids in the blood flowing from the adrenal glands in rats irradiated with minimum lethal doses of X-rays. The studies were run on 250 white male rats weighing 140-160 gm and kept on normal rations.

The animals were irradiated in the RUM-3 apparatus, at a voltage of 180 kV, current 15 mA, focal distance 40 cm, using filters of 0.5 mm Cu and 1 mm Al, power 35 r per minute, to a dose of 650 r. The animals all expired within 9-15 days. Corticosteroids were determined immediately after irradiation, and 1, 3, 15-17, 20-24, and 44-48 hours later.

The animals were subjected to the Cheboksarov operation. Under ether anesthesia, the operation required 20-30 minutes. Before blood was drawn, heparin was injected (50 units per 100 gm body weight), and blood was drawn over a period of 30 minutes. Corticosteroids in the efferent adrenal blood were extracted by the Bush method (9). Chromatography was done, using the toluol-methanol-water system (proportions 4:3:1) on paper obtained from the Leningrad factory; processing was done in 2 Normal acetic acid, water, 96 percent ethyl alcohol and methyl alcohol by the method of Axelrod (6). The chromatograph was examined under ultraviolet light with a UFS-1 filter; portions of paper containing corticosteroids were cut out and eluted in absolute methanol. Corticosteroids were determined quantitatively by ultraviolet ray absorption at a wave length of 240-243 mm in a SF-4 spectrophotometer. The results were evaluated statistically.

Determination of corticosteroids in small amounts of adrenal blood (6-10 ml) disclosed only one quantitatively-determinable corticosteroid - corticosterone. Tests on larger volumes of blood (62.7 and 39.3 ml) by this method also permitted quantitative determination of corticosterone only.

The content of corticosteroids in normal rats varied within limits of 241-392 γ per 100 ml (data from 83 rats). The maximal volume of blood obtainable from a rat weighing 140-160 gm during the 30-minute period of collection was 4.1 ml. In irradiated rats, corticosterone remained the only determinable hormone (quantitatively).

As is apparent from Table 1, immediately after irradiation, the content of corticosterone was reduced to 40 percent in comparison with the control; in 14 of 32 rats it could not be measured. Within one hour, the content of corticosterone did not change essentially, and remained at a low level, and in 10 animals of 25, none at all could be detected. Within three hours after irradiation, no corticosterone could be detected in the adrenal blood in any of the animals.

Table 1

Content of corticosterone in the adrenal blood of rats after irradiation

No. of experiment	Time	No. of tests	No. of rats	Average weight of rat in gm	Corticosterone content in γ per 100 ml of blood
1	Normal	12	83	157	268±17.0
2	Immediately after irradiation[1]	7	32	141	109±47.7
3	After one hour	9	25	156	106.8±31.0
4	After three hours	4	14	158	0
5	After 15-17 hours	3	12	155	169.7±9.9
6	After 20-24 hours	4	18	141	361.8±19.6
7	After 44-48 hours	5	22	144	224.4±24.0

[1] "Immediately after irradiation" - includes time of irradiation, operation, and withdrawal of blood.

Hence, in the course of the first three hours, the amount of corticosterone gradually declined to zero, after which it began slowly to rise. By 15-17 hours, the content of corticosterone had risen to 63 percent of the control level, and by 24 hours it had attained its highest levels, exceeding the control by 35 percent and returning to within the limits of the control only on the second day.

After processing the chromatographs of extracts of blood from irradiated rats with an alkaline solution of methanol, spots were found on them which were characteristic of steroids, with respect to their yellow fluorescence in ultraviolet light. These spots had an Rf of 0.30-0.33 and were found in chromatographs of blood extracts taken from rats at all periods following irradiation. Identification of the spot was not conclusive. Attempts were made to determine the content of corticosteroids in the blood of rats at more remote periods following irradiation - on the sixth and tenth days, but it proved impossible to collect blood for analysis.

As has already been mentioned, within three hours after irradiation corticosterone disappeared from the blood of the rats; during this time the weight of the adrenals was at a minimum. In order to clarify whether there was injury to the adrenal itself or to the pituitary regulatory function, the irradiated animals were given one unit of ACTH per 100 gm body weight in 0.2 ml physiologic saline solution just before collection of blood (Table 2).

Table 2

Content of corticosterone in the adrenal blood of rats following injection of ACTH within three hours after irradiation

No. of experiment	No. of animals	Weight of rats in gm	Weight of adrenals in mg	Volume of blood drawn, in ml	Corticosterone content in γ per 100 ml
1	5	150	11.2	7.8	291.8
2	5	146	13.26	6.4	271.8
3	4	145	11.8	7.1	308.0
4	4	148	10.8	5.7	605.0
5	4	179	12.2	4.8	304.0
6	5	165	12.1	10.2	Not measured
7	3	149	11.18	5.7	None detected

In five of the seven experiments, ACTH induced the secretion of corticosterone.

The secretion of corticosterone under the influence of ACTH reached levels seen in non-irradiated rats under conditions of stress induced by the operation.

It is obvious that the reduced concentration of corticosterone in the blood from the adrenals is due not to injury of the adrenals themselves but to disturbance of the pituitary regulatory function.

It should be noted that of 30 animals, injection of ACTH in three was not accompanied by secretion of corticosterone, and in five, corticosterone was found only qualitatively in the reaction with alkaline methanol.

The observations on the change in weight of the adrenals showed that these changes, although less pronounced, coincide in time with the variations in the content of corticosterone.

Table 3

Changes in weight of the adrenals in rats at various times after irradiation

No. of experiment	Time in experiment	No. of rats	Weight of adrenals in mg
1	Normal	80	12.56±0.28
2	Immediately after irradiation	30	11.96±0.36
3	After one hour	21	13.09±0.39
4	After three hours	14	11.18±0.42
5	After 15-17 hours	12	13.1 ±0.37
6	After 20-24 hours	18	13.7 ±0.85
7	After 44-48 hours	20	13.22±0.49

From the data of Table 3, it follows that within three hours after irradiation the weight of the adrenals had declined by an average of 11 percent in comparison with the normal, and within 24 hours it had exceeded the control by an average of nine percent; however, this difference is not statistically significant.

Conclusions

(1) The amount of corticosterone in the blood from the adrenal gland in rats, after irradiation, gradually declines to

zero by the third hour, and is then gradually restored. By 15-17 hours after irradiation, the content of corticosterone is 60 percent that of the control level, and within a day it has exceeded the control by 35 percent, becoming normalized on the second day.

(2) The injection of ACTH into the rats within three hours after irradiation, during the period in which corticosterone is not found in the adrenal blood, induces secretion of this hormone by the adrenals. The amount of corticosterone in the blood reaches control levels.

Bibliography

(1) Zhorno, L. Ya., "Distribution and Content of Ascorbic Acid in the Adrenals of Guinea Pigs during Radiation Sickness", Dissertation, Leningrad, 1956.

(2) Zakharoycheva, A. A., "Theses of Reports at the Scientific Meeting of the VIEE on the Problem of the 'Pituitary ", held 18-21 December 1957, Moscow.

(3) Orlova, L. V., Rodinov, V. P., Med. radiol., /Medical Radiology/, 1957, Vol 2, No 2, 54.

(4) Same authors, Pat. fiziol. i eksper. terapiya, /Pathological Physiology and Experimental Therapy/, 1957, Vol 1, No 4, 22.

(5) Tret'yakova, K. A., Probl. endokrin. i gormonoter., /Problems of Endocrinology and Hormone Therapy/, 1957, Vol 3, No 3, 72.

(6) Axelrod, L. R., J. Biol. Chem., 1953, Vol 205, p 173.

(7) Betz, E., "Contribution a l'etude du syndrome endocrinen provoque par l'irradiation total de l'organism", Paris, 1956.

(8) Bowers, J. L., Nelson, D. N., a. oth., J. Clin. Endocrinol., 1952, Vol 12, p 921.

(9) Bush, I. E., J. Endocrincl., 1953, Vol 9, p 9[illegible].

(10) French, A. B., Migeon, C. J., Samaels, L. T., Bowers, J. L., Am. J. Physiol., 1955, Vol 182, p 469.

(10) /sic/ Idem. J. Endocrinol. a. Metabolism, 1954, Vol 14, p 816.

(11) Nims, L. F. Sutton, E., Am. J. Physiol., 1954, Vol 177, p 51.

(12) Notter, G., Gemzell, C. A., Strahlentherapie, 1956, Bd. 99, S. 203.

(13) Patt, H.M., Swift, M. N., Turee, E. B., Johne, E. S., Am. J. Physiol., 1947, Vol 150, p 480.

(14) Wexler, B. C., Pencharz, R., Thomas, S. F., Proc. Soc. Exp. Biol. a Med., 1952, Vol 79, p 183.

(15) Idem. Am. J. Physiol., 1955, Vol 183, p 71.

Received
17 March 1958

END